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TITLE: Concurrent and Independent Genetic Alterations in Epithelial and Stromal Components of Breast Neoplasms: Implication for Tumor Development and Progression

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Rockville, Maryland 20849-0008

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Fort Detrick, Maryland 21702-5012

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U.S. Army Medical Research and Materiel Command
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13. ABSTRACT *(Maximum 200 Words)*

Our previous study on paraffin embedded tissues from patients with mammary carcinomas revealed a high frequency of loss of heterozygosity (LOH) in microdissected epithelial (EP) and adjacent or distant stromal (ST) cells. This study attempts to confirm previous findings on a larger scale and in a wider spectrum. Immunohistochemical and genetic analyses were performed on malignant and benign EP lesions with and without distinct ST alterations. Our findings show that morphologically comparable ST cells associated with malignant and benign lesions display a substantially different immunostaining patterns with antibodies to cell proliferation associated proteins, blood vessel components, and extracellular matrix molecules, and also show different frequencies and patterns of LOH at multiple chromosomal loci. ST cells from neither malignant nor benign lesions, however, display LOH or microsatellite instability with multiple DNA markers at chromosome 17p. These results are consistent with those of our previous studies, suggesting that [1] morphologically comparable ST cells in malignant and benign lesions are biofunctionally and genetically different and their genetic alterations correlate with those in their EP counterparts; 2] the biofunctions of ST cells may be independent of p53 gene regulation.

14. SUBJECT TERMS
Breast Cancer; Concurrent and independent genetic alterations; Epithelium - stroma interactions; Tumor development and progression

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Table of Contents

Introduction

A dynamic, reciprocal interaction between epithelial (EP) and mesenchymal (stromal) cells during embryogenesis is a well-recognized phenomenon, which determines the pattern and extent of the EP morphogenesis and cytodifferentiation (1-3). Recent studies have suggested that a similar interaction may also take place during the development and progression of mammary tumors (4-6). The molecular mechanism and detailed process of EP-stromal (ST) interactions during both neonatal and adult stages, however, have not been well elucidated. In addition, although a number of genetic alterations, including gene mutations, microsatellite instability (MI), and loss of heterozygosity (LOH), have been consistently detected in the EP component of both pre-malignant and malignant mammary lesions (7-9), the genetic profile of the ST component in these lesions remains essentially unexplored.

In a previous study (10), we evaluated the genetic profile of a type of "non-hyperplastic" flat intraductal carcinoma, also known as "clinging ductal carcinoma in situ", which is characterized by a single layer of mildly to severely atypical cells simply replacing the single layer of the native EP cells without appreciable proliferative or architectural alterations. In that study, morphologically normal ST cells at a distance (at least 15mm) from the EP component were microdissected to serve as normal internal controls. The ST cells in 22 of 25 (88%) cases displayed no genetic abnormality, while the ST cells in 3 cases (12%) showed LOH at multiple chromosomal loci. Those cases were excluded from that study because at that time we could not find adequate explanation in the literature. This surprising finding of LOH in ST cells prompted the initiation of a more detailed study. Paraffin embedded breast tissues from 11 female patients with intraductal or with invasive ductal carcinoma (IDC), and from 10 reduction mammoplasty specimen from normal women without radiological, or clinical and histopathological abnormality in their breasts, were selected for assessment of LOH. In each case, the normal appearing ST element (mainly fibroblasts, adipocytes, lymphocytes, and blood vessels) at a distance (at least 15mm) from, and close to the EP component, along with phenotypically different EP cells, were separately microdissected and placed in different tubes. The frequency and pattern of LOH among microdissected cells were compared after PCR amplification with a total of 12 DNA markers at

chromosomes 2p, 3p, 11q, 16q, and 17q. The LOH frequency in ST cells close to EP tumor cells ranged from 10-65% in intraductal carcinoma and from 20-75% in IDC. Furthermore, 10 of the 12 (83%) DNA markers showed LOH in the ST cells at a distance, ranging from 11–57 % of informative cases. In each case, the pattern and frequency of LOH in malignant EP cells were very similar to those in the ST component, and the EP and ST components in 8 (73%) of 11 cases displayed at least one identical LOH. In contrast, the frequency of LOH in clear-cut normal EP cells (33% or 4 of 12 markers) was substantially lower than that in normal appearing ST cells (83% or 10 of 12 markers) in cases with carcinoma. No LOH was detected in either EP or ST cells of the 10 reduction mammoplasty specimen used as normal controls. This study revealed a high frequency of LOH in normal appearing ST cells for the first time, suggesting that ST cells might be concurrently involved in the development or progression of mammary carcinomas, and that genetic alterations in ST cells may precede those in their EP counterparts. The manuscript of this study was published, with "highest priority", in Cancer Research (11), one of the most prestigious research journals.

The current study attempts to confirm our previous one using a larger number of cases and a wider spectrum of lesions. More importantly, as it has been suggested that the evolution and progression of human mammary carcinoma involves a sequential, multistep process, starting from transformation of normal cells to hyperplastic, then to in situ, and eventually to invasive and metastatic lesions, this study attempted to assess the correlation between EP and ST cells during various steps of the hypothesized multistep process of carcinogenesis. The hypothesis to be tested is that the ST component of a majority of mammary carcinomas plays a crucial role in preparing a receptive microenvironment for tumor development and progression, or more remotely both the EP and ST components are derived through a clonal growth of a common progenitor sharing a common immunohistochemical and genetic profile. The identification of genetic abnormalities in ST cells could facilitate the disclosure of the mechanism of the development and progression of EP tumors, and also might lead to the development of novel therapeutic modalities that specifically target ST cells, rather than the EP component of breast cancer.

Body (Materials and Methods)

The original purpose stated that: "The test will be accomplished by comparing the frequency and pattern of LOH, as well as the clonality between EP and ST cells. The test will compare carcinoma and benign lesions characterized by a distinctive stromal alteration with carcinomas and benign lesions lacking a distinctive stromal change, to determine:

[1] whether the EP cells and their surrounding ST cells display a similar frequency and pattern of LOH, and the same clonality;

[2] whether the frequency and pattern of LOH correlates with the EP phenotype;

[3] whether there is shared LOH in various patterns of intraepithelial neoplasms and invasive carcinomas" (see attached Appendix A)

To address these three issues, three corresponding experiments have been conducted:

[1] Paraffin embedded tissues from a total of 20 female patients with bilateral breast cancer had been retrieved from our files and processed for immunohistochemical and genetic analyses

[2] Paraffin embedded tissues from a total of 20 female patients with tubular carcinoma, which is a malignant lesion characterized by distinctive stromal alterations, and from 16 patients with infiltrating syringomatous adenoma, which is a benign lesion also characterized by distinctive stromal alterations, were retrieved from our files and processed for immunohistochemical and genetic analyses, including LOH and clonality analysis

[3] Paraffin embedded tissues from a total of 125 patients with various patterns of intraepithelial neoplasms and invasive carcinomas had been retrieved from our files and processed for immunohistochemical and genetic analyses.

Key research accomplishments

The original purpose listed the following five experimental procedures or objectives to accomplish the purposed project:

[1] Immunostaining sections with specific antibodies to highlight EP and ST cells;

[2] Microdissection of each EP phenotype and its surrounding stroma;

[3] DNA extraction from microdissected samples;

[4] PCR amplification of DNA extracts with fluorescent dye-labeled markers;

[5] Electrophoresis of PCR products and detection of signals with an automated DNA sequencer (see attached Appendix A).

All the listed procedures or objectives have been accomplished and the corresponding results have been analyzed prior to September 30, 2001.

Reportable outcomes

The main reportable outcomes can be summarized as follows:

[1]. EP tumor cells and their surrounding and distant ST cells display both concurrent and independent LOH at multiple chromosomal loci, while concurrent alterations are more frequently seen.

[2]. LOHs detected in ST cells appear to be clustered at chromosomes 3p, 11p, and 16q, but are rarely seen at chromosome 17p

[3]. Comparative genomic hybridization (CGH) analysis reveals an identical change, deletion of 13q, in both the EP and ST component of a cancerous breast.

[4]. Our preliminary studies reveal that the EP and ST component in some cases share the same clonality

[5]. Morphologically comparable ST cells associated with benign and malignant EP lesions display different immunohistochemical and genetic profiles, closely correlating with their EP counterparts.

[6]. The status of estrogen receptor (ER) expression in EP cells of intraepithelial neoplasms is closely associated with the integrity of the subjacent myoepithelial cell layer and the basement membrane.

For each of the three experiments corresponding to the three issues addressed in the proposal, the laboratory findings have been summarized, an abstract has been submitted to scientific conferences, and a manuscript is under preparation, which will be sent for publication in late 2001 or early 2002. The titles and the status of the three abstracts are as follows:

[1] Primary bilateral breast cancers display different LOH and CGH profiles in both epithelial and stromal components. Accepted for podium presentation at the 24th Annual San Antonio Breast Cancer Symposium" (see attached Appendix B).

[2] Morphologically comparable stromal cells associated with benign and malignant mammary epithelial lesions show different immunohistochemical and genetic profiles. Submitted to the 2002 Annual Meeting of US & Canadian Academy of Pathology (see attached Appendix C).

[3] **Focal loss of estrogen receptor (ER) expression in ER positive ductal intraepithelial neoplasia is associated with disruption of the immediate subjacent myoepithelial cell layer.** Submitted to

the 2002 Annual Meeting of US & Canadian Academy of Pathology (see attached Appendix D).

In addition, since this project required a great number of electrophoresis for PCR amplified products, an innovated protocol, which permits a significant reduction in electrophoresis time and reagent costs was developed in our Laboratory. The manuscript of this protocol has been published in **Electrophoresis 22:1915-1919, 2001**, a Med-Line listed journal (see attached Appendix E).

Conclusions

The frequency and pattern of LOH seen in our current study are consistent with those of our previous study (11). These findings appear to be sufficient and adequate to make a conclusion for each of the three questions addressed in our proposal (see attached Appendix A):

[1] The EP cells and their surrounding ST cells display a similar frequency and pattern of LOH, and the same clonality.

[2] The frequency and pattern of LOH correlates with the EP phenotype;

[3] There is shared LOH in various patterns of intraepithelial neoplasms and invasive carcinomas.

In addition, this study has expanded the spectrum of our previous findings in several respects:

[1]. For the first time, CGH analysis was performed in both EP and ST cells, and an identical CGH change was identified in both the EP and ST cells of a cancerous breast

[2]. Both the immunohistochemical and genetic profiles in morphologically comparable ST cells associated with benign and malignant EP lesions were assessed, and substantial differences were observed in these ST cells

[3]. Several chromosomal loci that display high frequencies of LOH in ST cells have been identified.

These findings appear to support our hypothesis that the EP and ST components of a majority of mammary carcinomas are derived through a clonal growth of a common progenitor. Even if the EP and ST cells were independently derived, the genetic and biofunctional alterations involved in carcinogenesis appear to result from dynamic, reciprocal interactions between the ST and EP cells. Ultimately, these findings suggest that ST cells play an important role in both the development and progression of the EP tumors. The elucidation of the mechanism and detailed process of these interactions may substantially facilitate the disclosure of the mechanism of the development and progression of epithelial tumors, and may also lead to development of novel therapeutic modalities that specifically target ST cells, rather than the EP component of breast cancer.

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2. Wiesen JF, Young P, Werb Z, Cunha GR. Signaling through the stromal epidermal growth factor receptor is necessary for mammary ductal development. Development 126:335-344, 1999
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11. Moinfar F, Man YG, Arnould L, Bratthauer GL, Ratschek M, Tavassoli FA. Concurrent and independent genetic alterations in the stromal and epithelial cells of mammary caicinoma: Implications for tumorigenesis. Cancer Res. 60:2562-2566, 2000

Appendices A-E

Concept Proposal Submission Form

This form has been set to accept 12 point Times New Roman font. For symbols use either (1) the Windows Character Map (under Accessories) or (2) from Word in a separate document, select Insert-Symbol...-(normal text). The use of other fonts is not recommended since the information may not be accurately transmitted.

☒ By checking this box, I attest that I (1) have a masters or doctoral degree (e.g., M.S., M.A., Ph.D., D.Sc., D.N.S., M.D., D.O., etc.) from an accredited institution; (2) have access to the necessary space and equipment to perform the proposed studies; and (3) am employed by an eligible institution (i.e., for-profit and nonprofit organizations, public and private, such as universities, colleges, hospitals, laboratories, companies, and agencies of local, state, and federal governments, including military laboratories). Therefore, I am eligible to submit this proposal.

1. Proposal Title (160 character limit): Concurrent and independent genetic alterations in epithelial and stromal components of breast neoplasms: Implications for tumor development and progression

2. Principal Investigator (PI):

   Last Name Tavassoli First Name Fattaneh MI A

3. Contact Information for PI:

   Organization Name American Registry of Pathology

   Department Name (if none, leave blank) Dept. of Gyn and Breast Pathology

   Street Address Line 1 6825 16th Street, NW

   Street Address Line 2 (if no line 2, leave blank) Building 54

   City Washington State DC Country USA Zip Code 20306-6000

   Phone 202-782-1600 Fax 202-782-3939

   E-mail tavassol@afip.osd.mil

4. Administrative Representative Authorized to Conduct Negotiations:

   Last Name King First Name Donald MI W

A

5. Contact Information for Administrative Representative Authorized to Conduct Negotiations:

Organization Name American Registry of Pathology

Department Name (if none, leave blank)

Street Address Line 1 6825 16th Street, NW

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City Washington State DC Country USA Zip Code 20306-6000

Phone 202-782-2102 Fax 202-782-4567

E-mail king@afip.osd.mil

6. Content Area of the Proposal
Please indicate up to two areas of emphasis to best describe your proposal. Enter the appropriate codes in the drop-down fields below.

01 Behavioral and Psychosocial Sciences
02 Cell Biology
03 Clinical and Experimental Therapeutics
04 Complementary and Alternative Medicine
05 Detection and Diagnosis
06 Endocrinology
07 Epidemiology
08 Genetics and Molecular Biology
09 Health Care Delivery
10 Immunology
11 Pathobiology
12 Primary Prevention
13 Radiation Sciences
14 Research Resources
15 Other, specify

Primary Proposal Content Area: 08 Secondary Proposal Content Area (Optional): 11

7. Will animals be used in the proposed work? No

8. Will human subjects be used in the proposed work? No

9. Will human anatomical substances, including primary and established cell lines, be used in the proposed work? Yes

10. Are laboratory experiments planned? Yes

11. PI gender (optional, select one): Female

12. PI ethnicity (optional, select one): White

If "Other" selected, specify _____

Data collected for questions 11 and 12 will be reported outside the Department of Defense only as grouped data without personal identifiers. Disclosure of this information is voluntary.

13. Budget Summary

Please adjust the indirect costs for your proposal below:

Direct Costs $50,000.00

Indirect Costs $4,000.00 (Please use the "Tab" key to go to the next field.)

Total Budget $54,000.00

14. Proposal Body

In the space below, please provide a clear and concise overview of the proposed work in 5,500 characters or less (~1 page). As appropriate, include the hypothesis, supporting rationale, objectives, relevance to breast cancer, and a general plan for how the project will be executed. Figures are not permitted and the use of tables is discouraged. Proposals will be reviewed by diverse panels of scientists, clinicians, and consumer advocates; therefore, applicants should consider the varied backgrounds of the reviewers when preparing proposals.

A dynamic, reciprocal interaction between epithelial and mesenchymal (stromal) cells during embryogenesis is a well-recognized phenomenon, which determines the pattern and extent of the epithelial morphogenesis and cytodifferentiation. Recent studies have suggested that a similar interaction may also take place during tumor development or progression. The molecular mechanism for the epithelial-stromal interaction, however, remains to be identified. While a number of genetic alterations, including microsatellite instability, loss of heterozygosity (LOH), and gene amplification, have been observed in benign and malignant epithelial lesions, similar genetic alterations in the stromal component of these lesions have not been studied extensively. Using the PCR technique with 12 DNA markers, one of our previous studies simultaneously examined DNA extracts from microdissected epithelial cells and stromal cells near and far away from the epithelial component of breast specimens from 11 cancer patients and 10 normal controls (1). A high frequency of LOH at chromosomal loci that harbor tumor suppresser genes was detected in both stromal and cancerous epithelial cells. While LOH at several loci was detected exclusively in the stroma, 8 of 11 (73%) cancer cases showed at least one identical LOH in both epithelial and stromal cells. None of the normal controls displayed LOH. Our findings raise the provocative possibility that the elusive "pluripotent stem cells" in the breast may give rise to both malignant epithelial cells and the associated supportive stroma, and also suggest that genetic alterations in mammary stromal cells may precede genetic changes in epithelial cells. The manuscript of our results has been accepted and ranked as "highest priority" for publication in "Cancer Research" at the earliest possible date (1).

We propose to confirm our previous observations on a larger number of cases. More importantly, since it has been documented that the evolution and progression of human cancer involves a sequential, multistep process, starting from the transformation of normal cells to hyperplastic disorders, then to in situ lesions, and eventually to invasive and metastatic carcinonomas, this study attempts to assess the correlation between epithelial and stromal cells during various steps of the hypothesized multistep process of carcinogenesis and tumor progression. The hypothesis to be tested is that the epithelial and stromal components of a majority of mammary carcinomas are derived through a clonal growth of a common progenitor; therefore, the epithelial cells and their surrounding stromal cells may share a common genetic

profile. The test will be accomplished by comparing the frequency and pattern of LOH, as well as the clonality between epithelial and stromal cells. The test will compare carcinomas and benign lesions characterized by a distincitive stromal alteration with carcinomas and benign lesions lacking a distinctive stomal change, to determine [1] whether the epithelial cells and their surrounding stromal cells display a similar frequency and pattern of LOH, and the same clonality; [2] whether the frequency and pattern of LOH correlates with the epithelial phenotype; [3] whether there is any shared LOH in various patterns of intraepithelial neoplasms and invasive carcinomas. The experimental procedures include: [1] Immunostaining sections with specific antibodies to highlight epithelial and stromal cells; [2] Microdissection of each epithelial phenotype and its surrounding stroma; [3] DNA extraction from microdissected samples; [4] PCR amplification of DNA extracts with fluorescent dye-labeled markers; [5] Electrophoresis of PCR products and detection of signals with an automated DNA sequencer. The protocol for each of the procedures (2-5), the tissue samples, and the facility to carry out the project are readily available in our department and the entire project can be accomplished within one year. The identification of genetic abnormalities in the stroma could facilitate the disclosure of the mechanism of tumor development and progression, and also may lead to development of novel therapeutic modalities that specifically target stromal cells, rather than the epithelial component of breast cancer.

15. References

No references are required for these submissions. Up to 5 references pertinent to the proposed study may be included in the boxes below. Limit each reference to 225 characters (~3 lines).

1.	Moinfar F, Man YG, Arnould L, Bratthauer GL, Ratschek M, Tavassoli FA. Concurrent and idependent genetic alterations in stromal and epithelial cells of mammary carcinoma...Cancer Res (In press, May 1, 2000)
2.	Moinfar F, Man YG, Bratthauer GL, Tavassoli FA. Loss of heterozygosity in mammary intraepithelial neoplasia---flat typt (Clinging carcinoma in situ)---Asimulator of normal mammary epithelium. Cancer (In press, May 1, 2000)
3.	Mooney EE, Man YG, Bratthauer GL, Tavassoli FA. Evidence that Leydig cells in Sertoli-Leydig cell tumors have a reactive rather than a neoplastic profile. Cancer 86:2312-2319, 1999
4.	Man YG, Moinfar F, Bratthauer GL, Tavassoli FA. Five unique strategoies for obtaining more valid gel images for LOH snd clonality assessments with an automated DNA sequencer. Diagn Mol Pathol (In press)
5.	Man YG, Schammel DP, Tavassoli FA. Detection of telomerase activity in microdissected breast leasions. Cell Vision 5:84-85, 1998

16. Principal Investigator Biographical Sketch

A biographical sketch of the PI must be submitted with the proposal but will not be considered in the peer or programmatic review process. A list of significant publications and a succinct summary of the investigator's professional experience in and/or potential for contribution to breast cancer research should be incorporated into the biographical sketch.

Biographical Sketch

Principal Investigator Name: Fattaneh A. Tavassoli

Position Title: Cairperson of Dept. of Gyn & Breast Path

EDUCATION/TRAINING: (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.) Include the name of the institution and location, degree earned (if applicable), years attended, and field of study. Limit to 800 characters.

Southwest MO State College, Cum Laude,	BS,	1968,	Chemistry
Medical School of St. Louis University, St. Louis	MD,	1972,	Medicine

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with your present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List in chronological order the titles, all authors, and complete references for all publications during the past 3 years and earlier publications pertinent to this application. If the list of publications in the last 3 years exceeds character limitations, select the most pertinent publications. Limit to 13,500 characters (~2.5 pages).

PROFESSIONAL EXPERIENCE

1972-1975 Resident, Dept of Surgical Pathology, Barnes Hopital, Washington U Medical School

1975-1976 Fellow, Dept of Gyn Pathology, St. John's Medical Center

1976-1992 Staff Pathologist and Vice Chairman, Gyn & Breast Pathology, AFIP

1992-1994 Chief, Gyn & Breast Pathology, Director of Research, Fairfax Hospital, VA

1995- Chairperson, Dept of Gyn & Breast Pathology, AFIP, ARP

Conultantship & Appointments

Clinical Assistant Professor of Pathology, Georgetown University Medical School, 1977

Lecturer and Instructor, Uniformed Services School of Health Sciences,1976-1983,985-1991

Clinical Professor of Pathology, Uniformed Services School of Health Sciences, 1986-1992

Consutant to National Institutes of Health, Dept of Surgical Pathology, 1987-present

Editorial Boards:

1. Member of the editorial board, Human Pathology
2. Member of the editorial board, The Breast Journal
3. Member of the editorial board, The International J of Gyn Pathology
4. Member of the editorial board, The international J of Surg Pathol
5. Ad hoc reviewer, Pathology, Research, & Practice
6. Ad hoc reviewer, Cancer
7. Ad hoc reviewer, Women's Health
8. Ad hoc reviewer, J of National Cancer Institute

Professional Society, Offices:

1. Member of Council on Anat Pathology, American Society of Clinical Pathologists, 1989-present
2. Member at Large, International Society of Gyn Pathologists
3. Abstract Review Board, International Academy of Pathology, 1990-1992

Publications:

A: Book

FA Tavassoli. Pathology of the Breast. 2nd Edition, 1999, Appleton & Lange

B: Computer expert system

FA Tavassoli. Pathology of the Breast. An interactive video expert system. 1990, Intellipath, Santa Monica

C: Representative journal articles (1998-present)

1. Moinfar F, Man YG, Arnould , Bratthauer GL, Ratschek M, Tavassoli FA. Concurrent and idependent genetic alterations in the stromal and epithelial cells of mammary carcinoma: implications for tumorigenesis. Cancer Res 60:2562-2566, 2000
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ABSTRACTS ARE DUE JUNE 1, 2001.

Use Typewriter to Fill in the Blanks.

2001 Official Breast Cancer Symposium Abstract Form

Type inside box below, using 11-point or larger Times or Times New Roman font. Box is 5 inches wide by 6 inches high (12.7 x 15.24 cm). Stay inside borders. Go to Page 3 for specific instructions.

1. PRESENTER NAME & ADDRESS:

Man Yan-gao MD., Ph.D

Last Name First Name Middle Initial Degree

Circle Choice: (Dr) Prof. Mr. Mrs. Ms. Miss

AFIP, Gyn & Breast Pathology

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I am a scientist-in-training, or a physician-in-training. My application (form page 7) is enclosed with this abstract in order to be considered for the award.

Primary bilateral breast cancers display different LOH and CGH profiles in both epithelial and stromal components

Man YG, Moinfar F, Shekitka KM, Stamatakos M, Lininger RA, Kuhls E, Bratthauer GL, and Tavassoli FA. Department of Gynecologic and Breast Pathology, Armed Forces Institute of Pathology and American Registry of Pathology, Washington, DC 20306-6000

One of our previous studies revealed that morphologically similar cells from two sides of bilateral primary breast cancers displayed a different pattern and frequency of loss of heterozygosity (LOH), suggesting that these might be independent lesions. This study attempted to confirm previous findings. **Methods:** The frequency and pattern of LOH and DNA copy numbers in microdissected epithelial (EP) and stromal (ST) cells from left and right lesions of 20 synchronous and metachronous breast cancers were compared, using PCR and comparative genomic hybridization (CGH) techniques. **Results:** A total of 147 LOH were detected in a total of 122 paired informative foci with a combination of 9 markers. Of 147 LOH, 82 (56%) were seen in the left and 65 (44%) in the right lesions. Of 122 paired foci, 97 (80%) showed unilateral and 25 (20%) displayed bilateral LOH. Of 42 paired, microdissected samples, 32 (76%) displayed more independent, while 4 (10%) showed more concurrent LOH. In 7 selected cases, CGH changes (gains or losses) were detected in the EP component in one side of three cases and in both sides of three cases, and loss of 11q was seen in the ST component in one side of two cases. CGH changes were distributed in 5 left and 4 right lesions, but none of cases that showed CGH changes displayed an identical pattern or frequency of changes in either EP or ST component of both breasts. **Conclusions:** These results are in favor of independent lesions in most primary bilateral cancers, and further suggest that ST cells are concurrently involved or even play initiative roles in development and progression of some breast cancers.

Completing this form does not register you to attend the Symposium. A Meeting Registration Form is provided on page 15.

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PRESENTER DISCLOSURE DECLARATION (Required with EACH abstract): It is the policy of The University of Texas Health Science Center at San Antonio Medical School to insure balance, independence, objectivity, and scientific rigor in all its sponsored educational programs. The presenters must disclose any real or apparent conflict of interest that may have a direct bearing on the subject matter of the program. This pertains to relationships with pharmaceutical companies, biomedical device manufacturers, or other corporations whose products or services are related to the subject matter of the presentation topic. The intent of this policy is merely to identify openly any potential conflict so that listeners may form their own judgments about the presentation with full disclosure of the facts.

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B

24th Annual San Antonio Breast Cancer Symposium

December 10-13, 2001

Sponsored by San Antonio Cancer Institute

August 1, 2001

ABSTRACT #24

General Session I: Monday 8:30 AM - 11:00 AM, Dec 10
General Session II: Monday 1:00 PM - 2:30 PM, Dec 10
General Session III: Tuesday 10:00 AM - 12 Noon, Dec 11
General Session IV: Wednesday 3:00 PM - 5:00 PM, Dec 12

Yan-Gao Man
AFIP
Gyn & Breast Pathology
6825 16th Street, NW
Washington, DC 20306-6000

RE: *Primary bilateral breast cancers display different LOH and CGH profiles in both epithelial and stromal components.*

Dear Dr. Man:

Your abstract referenced above has been accepted for slide presentation, General Session 3, at the 24th Annual San Antonio Breast Cancer Symposium. Instructions for a slide presentation are enclosed. Please follow them carefully.

Since yours is a General Session presentation, and two projectors are required for this very large meeting room, you must bring ***two sets of 35mm slides, and the sets must be identical.*** *If you plan to do a computer presentation,* ***notify Rich Markow*** *by answering the questions in the lower right hand box of the Slide Presenter Instructions form and faxing it back to him as soon as possible.*

If for any reason your presentation must be cancelled, notify the Symposium Coordinator as early as possible [E-mail Rmarkow@saci.org, FAX 210-949-5009, or phone 210-616-5912] before Friday, December 7. After that date, contact him at Marriott Rivercenter (210-223-1000). **Be sure to mention your assigned abstract number**. Failure to notify will result in activation of the No-Show Policy stated in the Abstract Submission Guidelines.

Don't forget to register to attend the meeting. You may register on line at www.sabcs.org. If you prefer fax or mail, a registration form is enclosed for your convenience. Pre-registration ends on October 31. **REGISTRATION IS CLOSED FROM NOVEMBER 1 UNTIL DECEMBER 9, 2001**. Your symposium materials will be given to you when you check in at SABCS in Conference Room 1-4.

For information on reserving a hotel room, go to the SABCS website at www.sabcs.org and click on the Housing button for instructions.

The final program booklet will be mailed in September. In the meantime, information is available on our website, which is updated periodically. Thank you for your abstract submission, and we look forward to your presentation.

Sincerely,
GARY C. CHAMNESS, PhD
Chairman, Abstract Selection Committee

24th Annual San Antonio Breast Cancer Symposium

Program Schedule

MONDAY, DECEMBER 10, 2001

8:00-8:10 WELCOME

Opening Remarks
Charles A. Coltman, Jr., MD

8:10-8:30 **The Role of Breast Cancer Advocates in Setting and Advancing a Research Agenda**
Frances M. Visco
National Breast Cancer Coalition
Washington, DC

8:30-11:00 GENERAL SESSION I

8:30 **1. A randomised placebo controlled trial to evaluate the effect of the bisphosphonate, clodronate, on the incidence of metastases and mortality in patients with primary operable breast cancer.**
Powles TJ, Paterson AH, McCloskey E, Ashley S, Tidy VA, Kanis JA, Pylkkänen L, Other Members of the International Multicentre Adjuvant Clodronate Group. Royal Marsden NHS Trust, London, United Kingdom; Tom Baker Cancer Centre and the University of Calgary, Calgary, Canada; University of Sheffield, Sheffield, United Kingdom; Leiras Clinical Research, Finland.

8:45 **2. Acute myeloid leukemia and myelodysplastic syndrome following doxorubicin-cyclophosphamide adjuvant therapy for operable breast cancer: the NSABP experience.**
Smith RE, Bryant J, DeCillis A, Anderson S. NSABP and Bristol-Myers Squibb.

9:00 **3. Delivering full planned dose on time chemotherapy while lowering the incidence of febrile neutropenia hospitalizations: Initial results from a prospective study providing Filgrastim support to high risk breast cancer patients.**
Rivera E, Erder HM, Fridman M, Brannan C, Frye DK, Hortobagyi GN. U.T. M.D. Anderson Cancer Center, Houston, TX; Amgen Inc., Thousand Oaks, CA; AMF Consulting Inc., Los Angeles, CA.

9:15 **4. Pilot trial of paclitaxel-herceptin adjuvant therapy for early stage breast cancer (E2198).**
Sledge GW, O'Neill A, Thor AD, Kahanic SP, Zander PJ, Davidson NE. Eastern Cooperative Oncology Group.

9:30 **5. The effect on primary tumor response of adding sequential Taxotere to Adriamycin and cyclophosphamide: preliminary results from NSABP Protocol B-27.**
NSABP, NSABP, Pittsburgh, PA.

9:45 **6. Serum HER-2/neu and response to the aromatase inhibitor letrozole versus tamoxifen.**
Lipton A, Ali S, Leitzel K, Demers L, Harvey H, Chaudri-Ross H, Brady C, Dugan M, Carney W. Hershey Medical Ctr., Hershey, PA; Novartis Pharma AG, Basel, Switzerland; Novartis Pharmaceuticals Corp., East Hanover, NJ; Oncogene Science, Cambridge, MA.

10:00 **7. The effect of tamoxifen on benign breast disease. Findings from the National Surgical Adjuvant Breast and Bowel Project (NSABP) Breast Cancer Prevention Trial.**
Tan-Chiu E, Costantino J, Wang J, Paik S, Butch C, Wickerham DL, Wolmark N. NSABP Operations Office, Pittsburgh, PA.

10:15 **8. The ATAC (Arimidex, Tamoxifen, Alone or in Combination) adjuvant breast cancer trial in post-menopausal women.**
Baum M, on behalf of the ATAC Trialists' Group.

10:30 **9. Final survival analysis of the double-blind, randomized, multinational phase III trial of letrozole (Femara®) compared to tamoxifen as first-line hormonal therapy for advanced breast cancer.**
Mouridsen H, Sun Y, Gershanovich M, Perez-Carrion R, Smith R, Chaudri-Ross H, Lang R, Brady C, Dugan M. Rigshospitalet, Copenhagen, Denmark; Chinese Academy of Medical Sciences, Beijing, China; Petrov Research Institute of Oncology, St. Petersburg, Russian Federation; Hospital Universitario de la Princesea, Madrid, Spain; South Carolina Oncology Assoc., Columbia, SC; Novartis Pharma AG, Basel, Switzerland; Novartis Pharmaceuticals Corp., E. Hanover, NJ.

10:45 **10. Letrozole (Femara®) is a more effective anti-proliferative agent than tamoxifen irrespective of ErbB1 and/or ErbB2 positive status: evidence from a phase III randomized trial of neoadjuvant endocrine therapy for postmenopausal women with estrogen receptor positive primary breast cancer.**
Ellis MJ, Jaenicke F, Llombart-Cussac A, Mauriac L, Miller WR, Evans DB, Brady C, Dugan M, Quebe-Fehling E, Borgs M. Duke University, Durham, NC; Universitaets Frauen-und Poliklinik, Hamburg, Germany; Instituto Valenciano de Oncologia, Valencia, Spain; Institut Bergonie, Bordeaux, France; West General Hospital, Edinburgh, United Kingdom; Novartis Pharma AG, Basel, Switzerland; Novartis Pharmaceuticals Corp., E. Hanover, NJ; The Letrozole Neoadjuvant Breast Cancer Study Group.

11:00-12:00 WILLIAM L. MCGUIRE MEMORIAL LECTURE

Endocrine Therapy for Breast Cancer—Past and Future
Nancy E. Davidson, MD
Johns Hopkins University School of Medicine
Baltimore, Maryland
Supported by an educational grant from Glaxo SmithKline

12:00-1:00 LUNCH [Ticket Required]

1:00-2:30 GENERAL SESSION II

1:00 **11. A common progenitor (adult stem) cell gives rise to both the gladular and myoepithelial cell lineages. A new cell biological concept as the basis of breast pathology.**
Boecker W, Moll R, van Diest P, Dervan P, Buerger H, Brandt B, Holland R, Poremba C, Diallo R, Buchwallow I. Institute of Pathology, University of Muenster, Muenster, Germany; Center of Pathology, University of Marburg, Marburg, Germany; Institute of Pathology, Free University Hospital, Amsterdam, Germany; Institute of Pathology, University of Dublin, Dublin, Ireland; Institute of Clinical Chemistry and Laboratory Medicine, University of Muenster, Muenster, Germany; Institute of Pathology, University of Nijmwegen, Nijmwegen, Netherlands.

1:15 **12. Risk of invasive local recurrence following therapy for ductal carcinoma in situ is a function primarily of time.**
Skinner KA, Sposto R, Helsper JT, Baril N, Silberman H, Waisman J, Silverstein MJ. Surgery, Preventive Medicine, and Medicine, Norris Cancer Center, University of Southern California, Los Angeles, CA.

1:30 **13. Endothelin-1 mediates pathological but not normal bone remodeling..**
Mohammad KS, Yin JJ, Grubbs BG, Cui Y, Padley R, Guise TA. Medicine/Endocrinolopgy, UTHSCSA, San Antonio, TX; Abbott Laboratories, Chicago, IL.

1:45 **14. Prognosis following local recurrence after conservative surgery and radiation therapy for early-stage breast cancer.**
Galper S, Blood E, Gelman R, Kohli A, Recht A, Harris J. Harvard Medical School, Boston, MA.

2:00 **15. The learning curve in sentinel node biopsy in breast cancer: results from the ALMANAC trial.**
Clarke D, Mansel RE, On behalf of ALMANAC trialists group. University Department of Surgery, Cardiff, Wales, United Kingdom.

2:15 **16. A randomized trial comparing axillary dissection and axillary radiotherapy for early breast cancer: 15 year results.**
Louis-Sylvestre C, Clough KB, Falcou M-C, Salmon RJ, Fourquet A, Vilcoq JR. Surgery, Curie, Paris, France.

2:30-5:00 MINI-SYMPOSIUM I

What's New in Surgery for Breast Cancer?
Anthony Lucci, MD, Co-Moderator
Baylor College of Medicine, Houston, Texas
And
Monica Morrow, MD, Co-Moderator
Northwestern University, Chicago, Illinois

2:30 **Introduction**

2:30 **Unresolved Issues in Sentinel Lymph Node Dissection for Breast Cancer**
Anthony Lucci, MD
Baylor College of Medicine
Houston, Texas

3:00 **Ductal Lavage and Ductoscopy: The Opportunities and the Limitations**
Seema Khan, MD
Northwestern University
Chicago, Illinois

3:30 **Four or five day brachytherapy instead of six weeks of external beam radiotherapy in the treatment of breast cancer**
Robert Kuske, MD
University of Wisconsin, Madison
Madison, Wisconsin

4:00 **Extending Breast Conservation**
J. Michael Dixon, MD
Edinburgh University Breast Unit
Edinburgh, Scotland

4:30 **Ablation Techniques for Breast Cancer**
Monica Morrow, MD
Northwestern University
Chicago, Illinois
Supported by an educational grant from AstraZeneca Pharmaceuticals

5:00-7:00 POSTER SESSION I & RECEPTION
(#101-180)

Detection / Diagnosis

Axillary/Sentinel Nodes	101-118
Biopsy Techniques	119-124

Risk and Prevention

Diet and Nutrition	125
Familial Breast Cancer	126-130
Prevention	131-139

Treatment

Breast Conservation	140-151
Radiation Therapy	152-159
Surgery	160-163

Tumor Cell Biology

Molecular Biology	164-167
Growth Factors/Inhibitors	168-174
Oncogenes/Tumor Suppressor Genes	175-180

TUESDAY, DECEMBER 10

7:30-9:30 POSTER SESSION II & CONTINENTAL BREAKFAST
(#201-280)

Detection / Diagnosis

Mammography/Imaging	201-213
Screening	214-219

Prognosis and Response Predictions

Predictive Factors	220-240

Risk and Prevention

Risk Factors	241-242
Adjuvant Therapy	243-252
DCIS	253-260
Cost-Effectiveness	261-265

Tumor Cell Biology

Angiogenesis	266-271
Antiestrogens	272-274
DNA Repair	275-277
Gene Therapy	278-280

9:30-10:00 PLENARY LECTURE I

Antiangiogenesis Therapy—where are we now?
Adrian L. Harris, MD, PhD
University of Oxford
Oxford, England, UK

10:00-12:00 GENERAL SESSION III

10:00 **17. Utilization of gene expression profiling to identify markers of disease recurrence in node-negative breast cancer.**
Roche PC, Mertens ML, Couch FJ, Ingle JN, Leontovich AA, Perez EA, Lillie J. Mayo Clinic, Rochester, MN; Millennium Predictive Medicine, Cambridge, MA.

10:15 **18. Improved survival benefit from Herceptin (trastuzumab) and chemotherapy in patients selected by fluorescence in situ hybridization.**
Mass R, Press M, Anderson S, Slamon D. Genentech, Inc., South San Francisco, CA; University of Southern California, Los Angeles, CA; Laboratory Corporation of America, Research, Triangle Park, NC; Division of Hematology and Oncology, UCLA School of Medicine, Los Angeles, CA.

10:30 **19. Level-I evidence for prognostic and predictive impact of uPA and PAI-1 in node-negative breast cancer provided by second scheduled analysis of multicenter Chemo-N0 therapy trial.**
Harbeck N, Meisner C, Prechtl A, Untch M, Selbmann H-K, Sweep F, Graeff H, Schmitt M, Jaenicke F, Thomssen C, for the Chemo N0 Study Group. Frauenklinik, Technische Universitaet Muenchen, Munich, Germany; Institut fuer Medizinische Informationsverarbeitung, Universitaet Tuebingen, Tuebingen, Germany; Frauenklinik Grosshadern, Ludwig-Maximilians-Universitaet, Munich, Germany; Universitaetsfrauenklinik Eppendorf, Hamburg, Germany; Dept. of Chemical Endocrinology, University Medical Center Sint Radboud, Nijmegen, Netherlands.

10:45 **20. Telomerase is a prognostic marker in breast cancer: high-throughput tissue microarray analysis of hTERT and hTR expression.**
Poremba C, Diallo R, Heine B, Sauter G, Boecker W. Gerhard-Domagk-Institute of Pathology, Westfaelische Wilhelms-University, Muenster, Germany; Institute of Pathology, Free University, Berlin, Germany; Institute of Pathology, University of Basel, Basel, Switzerland.

11:00 **21. Persistence of occult metastatic cells in bone marrow of breast cancer patients despite systemic adjuvant treatment.**
Janni WJ, Strobl B, Schindlbeck C, Rjosk D, Kentenich C, Stephan B, Harald S. I. Frauenklinik, LMU, Munich, Germany; Frauenklinik, TU, Munich, Germany.

11:15 **22. Persistence of solitary breast cancer cells in the secondary site: a possible source of turmor dormancy.**
Naumov GN, Kerkvliet N, Wilson SM, Nadkarni K, Morris VL, MacDonald IC, Groom AC, Chambers AF. University of Western Ontario, London, ON, Canada; London Regional Cancer Centre, London, ON, Canada.

11:30 **23. Genetic alterations in the progression of non-invasive to invasive breast cancer.**
Li Z, Tsimelzon A, Immaneni A, Mohsin SK, Hilsenbeck SG, Clark GC, Fuqua SA, Osborne CK, O'Connell P, Allred DC. Breast Center, Baylor College of Medicine, Houston, TX.

11:45 **24. Primary bilateral breast cancers display different LOH and CGH profiles in both epithelial and stromal components.**
Man YG, Moinfar F, Shekitka KM, Stamatakos M, Lininger RA, Kuhls E, Bratthauer GL, Tavassoli FA. Department of Gynecologic and Breast Pathology, Armed Forces Institute of Pathology and American Registry of Pathology, Washington, DC.

12:00-12:55 LUNCH [Ticket Required]

1:00-2:00 CASE PROBLEMS IN PRIMARY BREAST CANCER

2:00-5:00 MINI-SYMPOSIUM II

Translational Discoveries from the SPOREs
Suzanne AW Fuqua, PhD, Moderator
Baylor College of Medicine, Houston, Texas

2:00 **Introduction**

2:05 **A Hypersensitive Mutant Estrogen Receptor Alpha is Present in Most Invasive Breast Cancers**
Suzanne AW Fuqua, PhD
Baylor College of Medicine
Houston, Texas

2:30 **Strategies to Decipher SERM Stimulated Breast and Endometrial Cancer Growth**
V. Craig Jordan, PhD
Northwestern University
Chicago, Illinois

2:55 **Expression of Novel Transforming Oncogene GKLF/KLF4 in Breast Cancer**
J. Michael Ruppert, MD, PhD
University of Alabama at Birmingham
Birmingham, Alabama

3:20 **Roles of G1 Cyclins in Mammary Tumorigenesis**
Yan Geng, PhD
Dana-Farber Cancer Institute
Boston, Massachusetts

3:45 **Pleiotropin and its Receptor in Breast Cancer**
Anton Wellstein, PhD
Georgetown University Medical Center
Washington, DC

4:10 **Anti-HER2 Immunoliposomes for Targeted Drug Delivery**
John W. Park, MD
University of California, San Francisco
San Francisco, California

4:35 **Application of Methylated Gene Markers to the Early Detection of Breast Cancer in Ductal Lavage Fluid**
Saraswati Sukumar, PhD
Johns Hopkins University School of Medicine
Baltimore, Maryland

5:00-6:30 OPEN

6:30-8:30 THE BRINKER INTERNATIONAL AWARDS DINNER

The Susan G. Komen Breast Cancer Foundation and the 24th Annual San Antonio Breast Cancer Symposium cordially invite you to join us in the Marriott Rivercenter ballroom for dinner and presentation of the Brinker International Awards for Breast Cancer Research. [Ticket Required]

WEDNESDAY, DECEMBER 12

7:30-9:30 POSTER SESSION III & CONTINENTAL BREAKFAST
(#301-380)

Detection/Diagnosis

Circulating Markers	301-304
Marrow and Blood Micrometastases	305-309

Prognosis and Response Predictions

Prognostic Factors	310-341
Prognostic Factors – Methods	342-345

Outreach

Advocacy/Education	346-351

Treatment

Chemotherapy–General	352-365
Chemotherapy – Support	366

Tumor Cell Biology

Cell Biology	367-373
Drug Resistance	374-380

9:30-12:00 MINI-SYMPOSIUM III

Breast Imaging: Present and Future
Richard Elledge, MD, Moderator
Baylor College of Medicine
Houston, Texas

9:30 **Introduction**

9:30 **Seeing is Believing: Visualizing Gene Expression and Secondary Messengers by MRI**
Thomas J. Meade, PhD
California Institute of Technology
Pasadena, California

10:00 **Magnetic Resonance Elastography**
Don Plewes, MD
Sunnybrook Health Science Center & Women's College
Toronto, Ontario, Canada

10:30 **Breast Cancer Detection Using Functional Optical Spectroscopy and Imaging**
Bruce J. Tromberg, MD
University of California, Irvine
Irvine, California

11:00 **Positron Emission Tomography: Current and Future Applications**
Richard Wahl, MD
Johns Hopkins University School of Medicine
Baltimore, Maryland

11:30 **Using Magnetic Resonance Spectroscopy to Diagnose and Predict Therapeutic Response in Breast Cancer**
Michael Garwood, PhD
University of Minnesota
Minneapolis, Minnesota

12:00-12:55 LUNCH [Ticket Required]

1:00-2:00 CASE PROBLEMS IN ADVANCED BREAST CANCER

2:00-2:30 PLENARY LECTURE II

Post surgery radiation therapy for breast cancer: A review and critique of the overview analysis
Allen Lichter, MD
University of Michigan
Ann Arbor, Michigan

2:30-3:00 PLENARY LECTURE III

Predisposition to breast cancer : low penetrance genes - how and why
Prof. Bruce Ponder
Cambridge Institute for Medical Research
Cambridge, England, UK

3:00-5:00 GENERAL SESSION IV

3:00 **25. A hypersensitive estrogen receptor α is common in invasive breast cancer.**
Hopp TA, Van M, O'Connell P, Hilsenbeck SG, Mohsin S, Allred DC, Fuqua SA.
Breast Center, Baylor College of Medicine, Houston, TX.

3:15 **26. Use of hormonal contraceptives and breast cancer risk: "The Women's Lifestyle and Health Study".**
Kumle M, Weiderpass E, Braaten T, Adami H-O, Lund E. Institute of Community Medicine, University of Tromsoe, Tromsoe, Norway; Department of Medical Epidemiology, Karolinska Institutet, Stockholm, Sweden; International Agency for Reasearch on Cancer, Lyon, France.

3:30 **27. Epirubicin/Docetaxel (ET) versus 5FU/Epirubicin/Cyclophosphamide (FEC) combinations as first line chemotherapy in patients with metastatic breast cancer.**
Bonneterre JM, Dieras V, Tubiana-Hulin M, Bougnoux P, Bonneterre M-EA, Bendahmane B. Breast Cancer, Centre O. Lambret, Lille, France; Medical Oncology, Institut Curie, Paris, France; Medical Oncology, Centre R. Huguenin, Saint-Cloud, France; Radiation Therapy, CHU Bretonneau, Tours, France; Clinical Research Unit, Centre O. Lambret, Lille, France; Aventis, Paris, France.

3:45 **28. Circulating extracellular domain of HER2 can be correlated with clinical response to chemotherapy in locally advanced breast cancer.**
Stearns V, Yamauchi H, Singh B, Slack R, Hayes DF. Lombardi Cancer Center, Georgetown University Medical Center, Washington, DC.

4:00 **29. Inhibition of VEGF/KDR signaling by TSU-68 (SU6668), an oral anti-angiogenic agent, can synergistically enhance the anti-tumor activity of taxol; a new paradigm for breast cancer chemotherapy.**
Yonekura K, Basaki Y, Fujita H, Chikahisa L, Hashimoto A, Cherrington J, Shavwer L, Yamada Y, Kitazata K. Cancer Research Lavoratory, Taiho Pharmaceutical Co., Ltd., Hannno, Saitama, Japan; Sugen, San Francisco, CA.

4:15 **30. Therapeutic and chemopreventive effects of R115777, a farnesyl transferase inhibitor, on methylnitrosourea (MNU)-induced rat mammary cancer.**
Lubet RA, You M, Ruchon Y, End D, Wouters W, Christov K, Grubbs CJ. NCI, DCP, Ohio State Univ., Janssen Research Foundation, Univ. Ill. at Chicago, Univ. of Alabama at Birmingham.

4:30 **31. Dissection of cooperating genetic pathways involved in aggressive early onset breast cancer reveals mutually distinct roles for BRCA1 and HER2/neu genes.**
Olopade OI, Grushko TA, Hagos F, Adeyanju M, Adams AJ, Blackwood-Chirchir AM, Weber BL, Perou CM. Department of Medicine, University of Chicago, Chicago, IL; Department of Medicine, University of Pennsylvania, Pennsylvania, PA; Department of Genetics, University of North Carolina, Chapel Hill, NC.

4:45 **32. Coexpression of EGFr, HER2, HER3 and HER4 in primary human breast carcinoma.**
Witton CJ, Reeves JR, Going JG, Cooke TG, Bartlett JM. University Department of Surgery, Glasgow Royal Infirmary, Glasgow, United Kingdom; University Department of Pathology, Glasgow Royal Infirmary, Glasgow, United Kingdom.

5:00-7:00 POSTER SESSION IV & RECEPTION
(#401-480)

Detection/Diagnosis

Diagnostic Pathology	401-410

Epidemiology

Epidemiology	411-418
Racial Aspects	419-421

Treatment

Chemotherapy – High Dose	422-425
Chemotherapy – New Drugs and Formulations	426-442
Endocrine Therapy	443-459
Hormone Replacement Therapy	460-462

Tumor Cell Biology

Steroid Receptors	463-472
Antigens and Markers	473-475
Genetics	476
Tumor Biology	477-480

Supported by an educational grant from Pharmacia Oncology

THURSDAY, DECEMBER 13

7:30-9:30 POSTER SESSION V & CONTINENTAL BREAKFAST
(#501-580)

Detection/Diagnosis

Detection	501-505

Treatment

Neoadjuvant Chemotherapy	506-519
Antibody Therapy	520-530
Other Therapies	531-535
Patient Management	536-540
Psychosocial Aspects	541-544
Male Breast Cancer	545

Tumor Cell Biology

Apoptosis	546-550
Endocrinology	551-555
Immunology/Immunotherapy	556-562
Metastasis/Invasion	563-580

9:30-10:00 PLENARY LECTURE IV

Lobular carcinoma in situ: Much ado about nothing
Prof. Sunil R. Lakhani
Institute of Cancer Research
London, England, UK

10:00-12:30 MINI-SYMPOSIUM IV

Metastasis in Breast Cancer: Targets for Diagnosis and Treatment
Gregory R. Mundy, MD, Moderator
University of Texas Health Science Center
San Antonio, Texas

10:00 **Introduction**

10:00 **The Role of TGFβ in Breast Cancer Metastasis**
Theresa A. Guise, MD
University of Texas Health Science Center
San Antonio, Texas

10:30 **Cellular and Molecular Mechanism of Bone Cancer Pain**
Denis Clohisy, MD
University of Minnesota
Minneapolis, Minnesota

11:00 **Bisphosphonates and Tumor Progression in Osteotropic Breast Cancer**
Gabriel van der Pluijm, PhD
Leiden University Medical Center
Leiden, The Netherlands

11:30 **Angiogenesis and the Regulation of the Different Patterns of Metastatic Disease**
Michael S. O'Reilly, MD
MD Anderson Cancer Center
Houston, Texas

12:00 **The Role of IGF/NF-κB in Breast Cancer Metastasis to Bone**
Toshiyuki Yoneda, DDS, PhD
University of Texas Health Science Center
San Antonio, Texas

Supported by an educational grant from Aventis Oncology

12:30 ADJOURNMENT, 24th Annual San Antonio Breast Cancer Symposium

1:00-4:30 SATELLITE SYMPOSIUM

Optimal Therapy for Primary and Metastatic Breast Cancer: Emerging Standards and New Approaches

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For information, contact:
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Morphologically comparable stromal cells associated with benign and malignant mammary epithelial lesions show different immunohisto-chemical and genetic profiles

YG Man, KM Shekitka, M Stamatakos, L Tai, GL Bratthauer, PY Chen, FA Tavassoli
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Background: Our previous studies on paraffin embedded tissues from patients with mammary and cervical carcinomas revealed high frequencies of independent and concurrent loss of heterozygosity (LOH) in microdissected epithelial (EP) tumor cells and adjacent or distant stromal (ST) cells.

Design: To confirm previous findings on a larger scale and wider spectrum, and to determine whether morphologically comparable ST cells associated with benign and malignant EP tumors are bio-functionally and genetically different, the current study compared the immunostaining patterns and the frequency of LOH in EP and ST cells microdissected from infiltrating syringomatous adenomas and tubular carcinomas.

Results: Although the ST components in these two lesions were morphologically comparable, they displayed a substantially different immunostaining pattern to several antibodies, including antibodies to cell proliferation associated proteins, blood vessel components, and extracellular matrix molecules. The ST cells from these two lesions also displayed a substantially different frequency and pattern of LOH at multiple chromosomal loci, including 3p, 11p, 13p, and 13q. The ST cells from both lesions, however, displayed no distinct LOH or micro-satellite instability with multiple DNA markers at chromosome 17p.

Conclusions: These findings suggest that morphologically comparable ST cells associated with benign and malignant EP lesions are bio-functionally and genetically different, closely correlated with those in their EP counterparts. These findings also suggest that the bio-functions of ST cells in both lesions might not be directly subjected to the regulation of the p53 gene.

Acknowledgement: This study is supported by Congressionally Directed Medical Research Programs, The New Concept Award (DAMD17-00-1-0676) to Dr. Fattaneh A. Tavessoli and The Career Development Award (BC001187) to Dr. Yan-gao Man.

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Focal loss of estrogen receptor (ER) expression in ER positive ductal intraepithelial neoplasia is associated with disruptions of the immediate subjacent myoepithelial cell layer

YG Man, KM Shekitka, JS Saenger, L Tai, GL Bratthauer, PY Chen, FA Tavassoli
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Background: Our previous study using double immunostaining with antibodies to ER and smooth muscle actin (SMA) revealed patchy disruptions in the myoepithelial (ME) cell layer immediately subjacent to ER negative epithelial (EP) cells in mammary ducts with ostensibly EP proliferation.

Design: To confirm this finding on a larger scale, the same protocol was used to assess the association between ER expression and disruptions of ME cell layers on paraffin tissue sections from 125 patients with various grades of ductal intraepithelial neoplasia. The disruption of ME cell layers is defined as widening of a ME cell layer gap equal to the diameter of at least 3 EP cells in the cross section of a given duct. Focal loss of ER expression is defined as a significant reduction or complete loss of ER expression in a cluster of EP cells immediately overlying the disrupted ME cell layer, compared to strong ER expression in the remaining neoplastic cells within the same duct. The total number of the cross sections of ducts with proliferative changes was counted. All profiles with disrupted ME cell layers were photographed, and prints were made at a magnification of 400-800X for immunohistochemical and morphological assessments.

Results: Of the 125 cases, 62 (49.6%) showed disrupted ME cell layers; 246 (6.6%) disruptions were detected from 3,733 evaluated duct cross sections. Of the 62 cases with disrupted ME cell layers, 40 (64.5%) contained less than 4 and 22 (35.5%) displayed more than 4 disruptions. Of these disruptions, 225 (91.5%) from 59 cases were associated with focal loss of ER expression and 21 (8.5%) from 9 cases were subjacent to ER positive cells. The frequency and pattern of disruptions was generally independent of the size of ducts or the degree of neoplasia. The cells overlying the ME disruptions were generally morphologically indistinguishable from adjacent neoplastic cells within the same duct on routine H&E sections.

Conclusions: These findings suggest that focal loss of ER expression might play an important role in tumor progression and that double immunostaining with SMA and ER could assist in detection of incipient cancer invasion.

Acknowledgement: This study is supported by Congressionally Directed Medical Research Programs, The Idea Award (BC001187) and Career Development Award (BC001186) to Yan-gao Man, MD., Ph.D..

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Multiple use of slab gels in sequencing apparatus for separation of polymerase chain reaction products

Attempting to assess whether a decrease of the electrophoresis temperature could prevent or reduce the extent of gel well deformations, and whether the utilization of native polyacrylamide gels (without urea) could speed up the separation of polymerase chain reaction (PCR)-amplified products with an automated 377 DNA sequencer, denatured PCR products were subjected to electrophoresis in 6% native gels under 45°C. Results show that a decrease of the electrophoresis temperature from 51°C (recommended by the User's Manual) to 45°C substantially facilitates the preservation of gel wells, and that all PCR products tested migrate significantly faster in native than in denatured (with urea) gels of the same concentration. The combination of a 6% native gel and a lower (45°C) electrophoresis temperature permits multiple uses of a given gel with consistent results, consequently reducing the electrophoresis time and reagent costs.

Keywords: Electrophoresis / Native polyacrylamide gels / Polymerase chain reaction products / Automated DNA sequencer EL 4449

Attempting to reduce the electrophoresis time and reagent costs for evaluating polymerase chain reaction (PCR) amplified products, our recent study [1] assessed the mixture of multiple PCR products in the same well in a single run, with an automated 377 DNA sequencer and corresponding software (Perkin-Elmer, Foster City, CA, USA). Four PCR products with different molecular masses, ranging from 106 to 290 base pairs (bp), were pooled, the mixture and each of the corresponding pooled samples were separately loaded into 5–6% denatured polyacrylamide gels, and subjected to electrophoresis at 50°C according to the manufacturer's instructions [1]. Although the specific PCR product from each of four pooled samples was distinct, similar to those of corresponding samples that were individually loaded, the wells of gels were often deformed after electrophoresis, making repeated usage of the gel undesirable.This study attempted to determine whether a decrease of the electrophoresis temperature could prevent or reduce the extent of gel deformations, consequently allowing a given gel to be used multiple times. In addition, as a previous study [2] showed that the application of native gels facilitated the sizing of large DNA fragments (≈2,500 bp), this study attempted to determine whether the application of native gels could benefit the separation of smaller DNA molecules (<300 bp) of PCR-amplified products. Ultimately, this study attempted to determine whether the combination of a native gel and a decreased electrophoresis temperature could permit multiple uses of a gel and a further reduction of the electrophoresis time and reagent costs.

An automated 377 DNA sequencer with the corresponding software, the User's Manual, the DNA size standard (GENESCAN-500), and the gel loading buffer (10X Blue Dextran/EDTA) were obtained from Perkin-Elmer. PCR-amplified products with fluorescent dye labeling were obtained from our previous studies [1, 3–6]. Polyacrylamide gel solution (40% acryl/bis 19:1) and urea were purchased from Bio-Rad (Hercules, CA, USA). TEMED, formamide, and ammonium persulfate were bought from Amresco (Solon, OH, USA). Denatured gels (with urea) were prepared strictly following the formula provided by the User's Manual, and native gels at the same concentrations were prepared using the same formula but without urea. For a direct comparison, denatured and native gels at the same concentration were prepared in the same plate. This was accomplished by adding two additional spacer bars to the center of the gel plate to create two isolated compartments, which allow two different gel formulations to be poured simultaneously into the same plate apparatus.

Samples were prepared for electrophoresis, according to the User's Manual and our previously published protocol [1]. Briefly, each PCR product or a mixture of 3–4 PCR products was mixed with the loading buffer containing

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formamide. The mixtures were incubated for 4 min at 95°C, and then placed in ice for loading. To assess the impact of a decreased electrophoresis temperature on the preservation of the gel wells, different gels of the same concentration and formulation were subjected to electrophoresis under the temperature recommended by the User's Manual (51°C) and a decreased temperature (45°C). After each electrophoresis, 100–150 μL of the loading buffer were added to the slot to elucidate the wells. The averaged number of nondeformed wells in 3–4 separated runs under each temperature was compared. To evaluate the potential benefits of a native gel over a denatured gel of the same concentration, the identical PCR mixtures were subjected to electrophoresis in each gel type. The averaged relative mobilities of the identical PCR products and the separation time in 3–4 separated runs of each gel type were statistically compared with the Student's *t*-test. To determine whether a combination of a native gel and a decreased temperature may permit multiple uses of a gel and a reduction of the electrophoresis time, a 6% native gel was subjected to electrophoresis multiple times under 45°C for the identical PCR mixtures. The relative mobility and separation time of the identical PCR products on gels used multiple times were statistically compared to those in gels used only once, and also to those in gels under the conditions recommended by the User's Manual. In 4% denatured and native gels, only 26 and 38% of the wells were not deformed under 51°C and 45°C, respectively. In 6% denatured and native gels, however, 44 and 94% of the wells remained unimpaired under 51 and 45°C, respectively, suggesting that a decreased electrophoresis temperature facilitates the preservation of the wells of 6% gels.

The averaged separation time in denatured and native gels at both 51°C and 45°C were listed in Table 1. The separation time for each of the PCR products assessed at both 51°C and 45°C was significantly shorter ($p < 0.01$) in both 4 and 6% native gels than in the corresponding denatured gels. Figures 1a and b show the band pattern image of three PCR products sized from 100 to 217 bp in 6% denatured and native gels on the same plate under the same electrophoresis temperature. Each of the three PCR products and the DNA size standard appeared to migrate substantially faster in the native gel than in the denatured gel. This was confirmed by the statistical comparison of the relative mobilities, which revealed that the migration of all three PCR products was indeed significantly faster ($p < 0.01$) in the native than in the denatured gel (Table 2). One conceivable drawback of native gels is the proximity of the bands which, while still sharply defined, are confined to a more limited expanse of the gel. This drawback, however, has little impact on the interpretation of the results, as the band pattern image can be transformed into a densitometric graph, which is more objective and easier to interpret (while far more time-consuming). As shown in Fig. 1c and 1d, each of the two alleles of the PCR product is clearly elucidated as a distinct curve in both native and denatured gels, indicating that the same PCR product can be separated in both gels.

The combination of a 6% native gel and a decreased electrophoresis temperature permitted multiple uses of a given gel, consequently reducing the electrophoresis time

Table 1. Comparison of the averaged separation time between denatured and native gels at 51°C and 45°C

Gel type	Separation time (min) for PCR products of different sizes (bp)		
	100–112	142–158	210–227
4% Denatured at 51°C	37	44	57
4% Native at 51°C	25	30	35
4% Denatured at 45°C	39	48	63
4% Native at 45°C	30	34	41
p	<0.01	<0.01	<0.01
6% Denatured at 51°C	53	68	91
6% Native at 51°C	34	44	56
6% Denatured at 45°C	59	75	101
6% Native at 45°C	37	45	58
P	<0.01	<0.01	<0.01

Identical PCR mixtures were subjected to electrophoresis in each gel type at 51°C and 45°C. The averaged separation time in 3–4 separated runs of each gel type at each temperature was statistically compared with the Student's *t*-test.

Table 2. Comparison of relative mobilities of PCR products on 6% denatured and native gels on the same plate under the same electrophoresis condition

Gel type	Relative mobilities of PCR products with different sizes (bp)		
	100–112	142–158	210–227
6% Denatured	0.60–0.57	0.43–0.40	0.18–0.15
6% Native	0.82–0.80	0.74–0.73	0.63–0.61
P	<0.01	<0.01	<0.01

Two isolated compartments were created on the same gel plate by adding two additional space bars to the center of the plate, and 6% denatured and native gels were simultaneously poured into each compartment. Three identical PCR products and a DNA standard were subjected to electrophoresis in each gel under the same condition. The relative mobilities of the same PCR products were statistically compared with the Student's *t*-test.

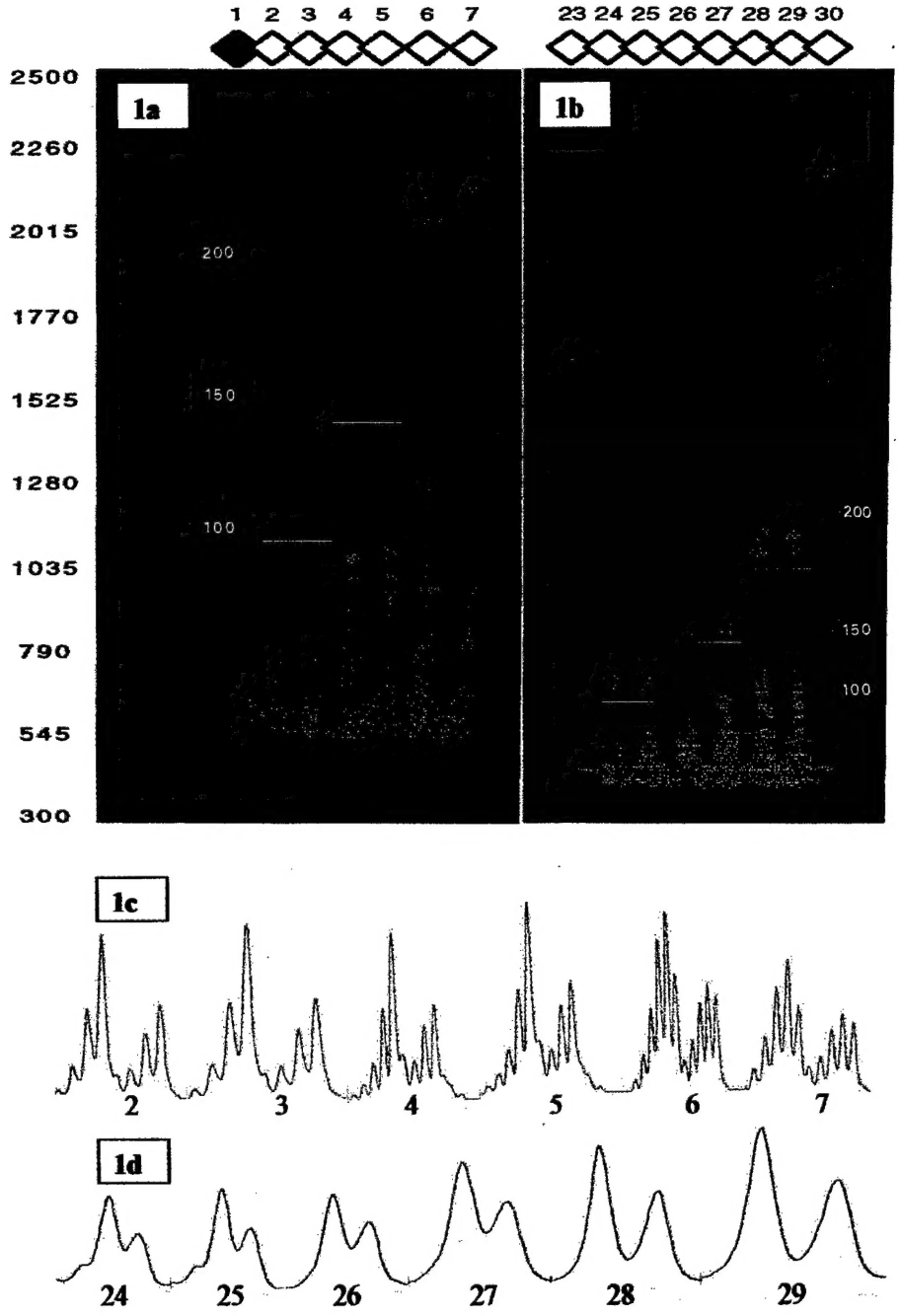

Figure 1. Separation of PCR products on 6% denatured and native gels on the same plate under the same electrophoresis condition. Two isolated compartments were created on the same gel plate by adding two additional space bars to the center of the plate, and 6% denatured and native gels were simultaneously poured into each compartment. Three identical PCR products and a DNA standard were subjected to electrophoresis in each gel under the same condition. Band pattern and densitometric gel images were prepared based on the User's Manual and our published protocol. The specific PCR products were underlined. The numbers on the left of each figure represent the time/molecular weight scale. The red bands represent the DNA size standard, containing 16 ROX-labeled DNA fragments, ranging from 35 to 500 base pairs. (a) The band pattern image on the denatured gel. (b) The band pattern image on the native gel. (c) The densitometric image on the denatured gel. (d) The densitometric image on the native gel.

and reagent costs. In most cases, a given 6% native gel could be continuously used 3–4 times under 45°C. Although slightly deformed wells were occasionally observed after each run, they could usually be fixed with the gel cleaning apparatus designed in our laboratory [1]. Table 3 shows the comparison of the averaged separation time of three PCR products in 3–4 separate sets of electrophoresis in a 6% native gel that was used four times. The averaged separation times among four different runs were almost identical.

Table 4 shows the comparison of the corresponding relative mobilities. Again, the mobilities of all the PCR products among different runs were not statistically different ($p > 0.05$). Figures 2a–e show the band pattern images of four sequential runs of the identical PCR mixtures in the same gel, and of the first run in a gel that was prepared and subjected to electrophoresis strictly based on the User's Manual. The shape and intensity of DNA bands among four sequential runs were very similar to each other, and also similar to those in the comparing gel,

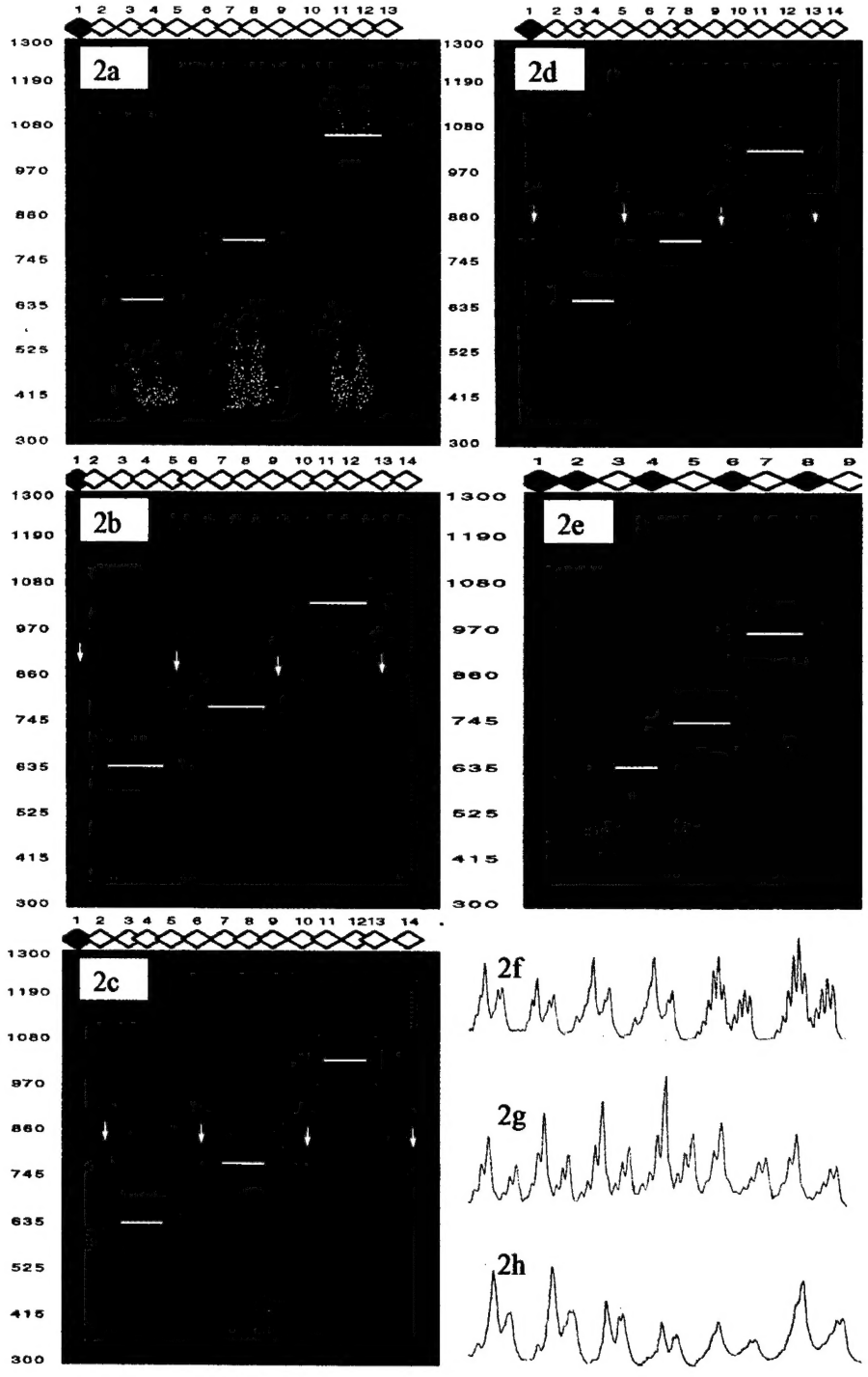

Figure 2. Comparison of the gel images on gels used different times. Three identical PCR products and a DNA size standard were subjected to electrophoresis in a 6% native gel four times at 45°C and in a 4% denatured gel once at 51°C. Band pattern and densitometric gel images were prepared based on the User's Manual and our published protocol. The specific PCR products were underlined. The numbers on the left of each figure represent the time/molecular weight scale. The red bands represent the DNA size standard containing 16 ROX-labeled DNA fragments, ranging from 35 to 500 bp. (a)–(d) The band pattern images on the gel during the first to fourth runs. Note the persisted large DNA fragments from the previous run (arrows). (e) The band pattern image on a 4% denatured gel at the first run at 51°C. (f)–(g) The densitometric images on the gel at the first and fourth run. (h) The densitometric image on the 4% denatured gel at the first run.

which was conformed by the raw data collected by the detection system during the electrophoresis process (data not shown). Figures 2f–h show the densitometric graphs of the first and the fourth run in the same gel, and of the first run in the comparing gel. Again, the height of the peak and the curve pattern in the fourth run was similar to those in the first run of both the same gel and the comparing gel.

In summary, this study has demonstrated that a decrease of electrophoresis temperature from 51 to 45°C facilitates the preservation of gel wells. This study has also shown that native gels could serve as a rapid tool for a preliminary run that would quickly show the outcome of PCR amplifications. The combination of a 6% native gel and a decreased electrophoresis temperature permits multiple uses of a given gel with consistent

Table 3. Comparison of the averaged separation time of PCR products with the same size in 6% native gels used different times

Number of usage	Averaged separation time (min) for PCR products with the same size (bp)		
	100–112	142–158	210–227
1	37.0	46.0	58.5
2	37.0	45.0	58.0
3	37.0	45.5	58.0
4	36.5	45.0	57.5
P	>0.05	>0.05	>0.05

A 6% native gel was subjected to electrophoresis four times under 45°C for the same PCR mixtures. The averaged separation time, in 3–4 separate sets of electrophoresis, of the PCR product with the same size at different runs was statistically compared with the *F*-test.

Table 4. Comparison of the relative mobilities of PCR products in 6% native gels used once and multiple times

Number of usage	Relative mobility of PCR products with the same size (bp)		
	100–112	142–158	210–227
1	0.60–0.56	0.43–0.40	0.15–0.10
2	0.59–0.57	0.43–0.41	0.15–0.10
3	0.61–0.58	0.45–0.42	0.17–0.13
4	0.61–0.58	0.46–0.43	0.18–0.15
P	>0.05	>0.05	>0.05

A 6% native gel was subjected to electrophoresis four times under 45°C for the same PCR mixtures. The averaged relative mobility, in 3–4 separate sets of electrophoresis, of the PCR products with the same size at different runs was statistically compared with *F*-test.

results, consequently reducing the electrophoresis time and reagent costs.

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